PROGNOSTIC FACTORS IN GASTROINTESTINAL STROMAL TUMOURS

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CERTIFICATE

I hereby certify that this is the bonafide work done by **Dr. AQIB K. SHAICK** who is appearing for **MCh Surgical Oncology** (branch VII) final examination in February 2008, under my guidance in the College of Oncological Sciences, Cancer Institute (WIA), Adyar Chennai.

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CONTENTS

INTRODUCTION

Gastrointestinal stromal tumours (GIST) are the most common mesenchymal tumours of the digestive tract. Most gastrointestinal soft tissue neoplasms, previously classified as leiomyomas, schwannomas, leiomyoblastomas or leiomyosarcomas, are today classified as GIST on the basis of molecular and immunohistological features. They originate from gastrointestinal pacemaker cells and are characterised by over-expression of the tyrosine kinase receptor KIT. Overall 5-year survival after surgical resection of GIST is approximately 60%. However, these tumours span a wide clinical spectrum from benign to highly malignant. Prognostic factors have recently been identified for GIST and include tumour size, mitotic rate and other minor factors. At present, surgery is the standard treatment for primary resectable GIST. Benign GIST have an excellent prognosis after primary surgical treatment, with over 90% 5-year survival. While recurrent or malignant GIST, which are resistant to radiotherapy and chemotherapy, had until recently an extremely poor prognosis even after surgical resection, with median survival of 12

months. The development of a tyrosine kinase inhibitor has changed the management of unresectable malignant cases. This new tyrosine kinase inhibitor, imatinib mesylate, which inhibits the c-kit receptor, has proved highly effective against GIST and has improved survival in metastatic GIST.

AIMS & OBJECTIVES

1. To find out the actual number of Gastrointestinal stromal tumours treated in our institute based on c-kit positivity.
2. To identify the prognostic factors influencing recurrence and survival.
3. To identify subgroup of patients, who might benefit from adjuvant therapy.

MATERIALS & METHODS

All the case records of patients who were diagnosed to have GIST (on the basis of c-kit positivity) were analysed individually. Case records of all the patients who were diagnosed to have intraabdominal sarcoma from the year 1999 to june 2007 were scrutinized. Paraffin blocks and slides of the above patients were retrieved from pathology dept. IHC study for c-kit were performed on the slides. All the c-kit positive cases were also included in the study. Of the 32 patients with GIST only 31 were available for analysis.

Data was analysed using SPSS 10.0.1 structured package.

Survival was calculated by life table method.

Comparison of survival by different categories were done by log rank test.

REVIEW OF LITERATURE

Gastrointestinal stromal tumours (GISTs) are rare tumours of the gastrointestinal tract, mesentery, and omentum. However, malignant GIST is the most common sarcoma of the gastrointestinal tract and accounts for about 5% of all sarcomas.1 The estimated annual incidence is 10–20 cases per million, of which 20–30% are malignant.2 However, these estimates may need to be revised after the recent clearer definition of diagnostic criteria for GIST.3 Clinical, histopathological, ultrastructural, and molecular-biological findings, have made clear that GIST is completely separate from leiomyoma and leiomyosarcoma, which were previously thought to be the most common types of softtissue neoplasms in the gastrointestinal tract. Recent studies suggest that true gastrointestinal-tract leiomyomas and leiomyosarcomas are rare.2

The term GIST was first used by Mazur and Clark in 1983 to describe gastrointestinal non-epithelial neoplasms that lacked the

immunohistochemical features of Schwann cells and did not have the ultrastructural characteristics of smooth-muscle cells.4 The discovery of gain-of-function mutations in the *KIT* proto-oncogene in GISTs by Hirota and colleagues in 1998 was of crucial importance in terms of the genesis and classification of these tumours.5 This finding led to the development of rational, molecularly targeted therapy of GISTs with the KIT-receptor tyrosine-kinase inhibitor, imatinib mesylate (formerly known as STI571).

The KIT protein is the transmembrane receptor for the cytokine known as stem-cell factor (SCF); the intracytoplasmic portion of KIT functions as a tyrosine kinase. At present, GISTs are defined as spindle-cell, epithelioid, or occasionally pleiomorphic mesenchymal tumours of the gastrointestinal tract that express the KIT protein.2,3 The KIT protein is often detected clinically by immunohistochemical assays for CD117 antigen. The definition of KIT-negative GISTs remains a focus of research, but for clinical purposes the important point is that the vast majority of GISTs express KIT.

CLINICAL FEATURES

Most studies of the clinicopathological entity referred to as GIST before the year 2000, are likely to include a group of patients with true GISTs as well as other histological subtypes of spindle-cell sarcoma such as leiomyosarcoma. GISTs occur in both sexes at a similar frequency, but some data show male predominance.2,6 The median age at diagnosis is about 60. GISTs are occasionally found in young adults, but they are very rare in children.2 Nearly all GISTs arise as a result of a somatic mutation, but rare familial cases associated with mutated *KIT* have been identified.

The hereditary forms are characterised by the presence of multiple tumours and in some cases hyperpigmentation of the skin and the mucous membranes, systemic mast-cell disease, multiple naevi, urticaria pigmentosa, and diffuse spindle-cell hyperplasia in the myenteric plexus layer of the gastrointestinal tract.7–9 GISTs may also be a feature of the Carney triad, a very rare syndrome of unknown cause affecting mainly young women.

The triad includes gastric stromal sarcoma (generally epithelioid type), extra-adrenal paraganglioma, and pulmonary chondroma. Familial occurrence has been suggested for the Carney triad, but no detailed molecular genetic mechanism is known.10 A pathogenetic relation has also been suggested between neurofibromatosis type 1 (von Recklinhausen's disease) and GISTs because of the high frequency of nonrandom association of these diseases.11 However, the vast majority of GISTs are sporadic, and predisposing factors are unknown.

GISTs are most commonly found in the stomach (40–70%), but they can occur in all other parts of the gastrointestinal tract. About 20–40% of GISTs arise from the small intestine, and 5–15% from the colon and rectum.1,2,12,13 GISTs can also be found in the oesophagus (<5%), omentum (<5%), mesentery, or retroperitoneum.2,3,12 They typically grow in an endophytic way parallel to the bowel lumen, commonly with overlying mucosal necrosis and ulceration, and they vary in size from a few millimetres to 40 cm in diameter.12 Larger, high-grade GIST lesions, can be necrotic and haemorrhagic and show more mucosal ulceration than smaller GISTs, which might have been diagnosed in the past as purely benign lesions. Many GISTs are well confined by a very

thin surrounding pseudocapsule.14 Over 95% of patients present with a solitary primary tumour; in 10–40% of cases these tumours directly invade the surrounding organs.15,16

Many small GISTs are discovered incidentally during endoscopy or laparotomy done for other reasons such as submucosal or subserosal nodules, or during imaging examinations. At presentation, the most common symptoms of GISTs are vague abdominal discomfort or pain, presence of a palpable abdominal mass, feeling of abdominal fullness, and secondary symptoms resulting from tumour bleedingand associated anaemia. GISTs can also cause altered bowel function, bowel obstruction or perforation, dysphagia, and fever. Duodenal GISTs occasionally cause obstructive jaundice. GISTs are commonly discovered during emergency surgery for unexpected perforation of the gastrointestinal tract and consequent intra-abdominal blood loss.17 15–50% of GISTs present with overtly metastatic disease.1, 16, 18.

A peculiar feature of GISTs is that the great majority of recurrences are solely intra-abdominal. Macroscopic extraabdominal metastases are uncommon even in advanced disease, and they rarely

occur in the absence of intraabdominal recurrence. This feature contrasts with true leiomyosarcomas of the abdomen and gastrointestinal tract, which commonly give rise to pulmonary metastases.6,19 40–80% of GISTs recur despite histopathologically complete tumour resection. The most common sites of metastases are the peritoneum and the liver,1,6 whereas regional lymph-node metastases are extremely rare.1,20 In one review of 60 patients with recurrent GIST, local recurrence occurred in 76% of patients, half of whom had synchronous liver metastases, 15% liver metastases, and 7% peritoneal metastases.21 None had extra-abdominal metastases at first recurrence.

Peritoneal metastases are most probably a result of tumourcell seeding from the primary tumour directly into the peritoneal cavity. Similarly, liver metastases most probably result from haematogenous seeding into the portal vein.

Histopathology and immunohistochemistry

On histolgical analysis most GISTs look fairly benign, which is surprising in view of the malignant potential of the disease. However, the

histological appearance of GISTs can vary greatly among patients, and their malignant potential ranges from clinically benign tumours to aggressive cancers. The spindle-cell variant of malignant GIST (70%) corresponds to tumours previously classified as leiomyosarcoma, and many of the epithelioid or round-cell variants (30%) were previously thought to be leiomyoblastoma. Most tumours previously diagnosed as gastrointestinal autonomic nerve tumours (GANTs) are in fact GISTs.2,3,22 GANTs have been described as cells with axon-like cytoplasmic processes and synapse-like structures (seen on electron microscopy), with dense core granules and intercellular fibrils.

Tumours previously diagnosed as GANTs have subsequently been found to express KIT and to harbour essentially identical KIT mutations to GISTs.23 Also, electron microscopy shows that a substantial proportion of GISTs have similar ultrastructural features to GANTs. Thus, GANT should be regarded as a type of GIST and no longer be classed as a separate entity.3It is important to differentiate between GISTs, which constitute about 80% of gastrointestinal mesenchymal tumours, and the less common gastrointestinal nonepithelial neoplasms, leiomyoma, leiomyosarcoma (10–15% of mesenchymal tumours),

schwannomas (5%), and other malignant disorders such as melanomas, so that appropriate clinical decisions can be made (table 1). GISTs characteristically stain strongly for the CD117 antigen, an epitope of the KIT-receptor tyrosine kinase. Smooth-muscle neoplasms (leiomyoma and leiomyosarcoma), neurogenic tumours (schwannomas), and desmoid fibromatoses typically do not show this positive expression of CD117.2,3,22

Thus, CD117 immunostaining is an important method for diagnostic distinction. Typically, in GISTs, KIT is widespread throughout the entire tumour and is highlighted by cytoplasmic staining, sometimes showing a dot-like ‘golgi’ pattern.3 60%–70% of GISTs stain for CD34, a sialylated transmembrane glycoprotein also found in haemopoietic progenitor cells and endothelial cells.3,5,22,24 Up to 40% of GISTs are also positive for smooth muscle actin (SMA).

They rarely express desmin, an intermediate filament protein typical of muscle, or S100, a neural (schwann) cell marker, and they are negative for neurofilaments and glial fibrillary acidic protein.2 By contrast, leiomyosarcomas are positive for SMA and desmin but negative for KIT, and schwannomas are positive for S100 but negative

for CD117.3,22 Overall, strong KIT expression in the absence of smooth-muscle differentiation-related proteins is characteristic for GISTs, and these features aid the differential diagnosis between GISTs and most other types of gastrointestinal mesenchymal tumours.

KIT expression is not limited to GISTs, but some other neoplasms may stain positively for KIT in immunohistochemical assays. These tumours include a subset of soft-tissue sarcomas such as Ewing's sarcoma and angiosarcoma, as well as other neoplasms such as melanoma, small-cell lung cancer, adenoid cystic carcinoma, ovarian carcinoma, sinonasal natural-killer/T-cell lymphoma, anaplastic large-cell lymphoma, acute myelogenous leukaemia, seminoma, neuroblastoma, and mastocytomas.

However, these tumours are rarely included in the differential diagnosis of GISTs.2,25,26 Immunopositivity for KIT does not necessarily indicate KIT activation or the presence of a *KIT* mutation, and there is, as yet, little reason to believe that these other tumours would show a response to imatinib mesylate.

Table 1. Differential diagnosis of gastrointestinal stromal tumours

	Histology	KIT	SMA	Desmin	S100	CD34	Cytogenetics and molecular genetics
GIST	Spindle cell or epithelioid; fibrillary/syncytial cytoplasm; most are monomorphic	+	+ (30–40%)	+ (1–2%)	+ (about 5%)	+ (60–70%)	Monosomies 14 and 22; deletion of 1p; KIT mutations in up to 90%
Smooth-muscle neoplasm	Most are spindle cell; variable atypia; well-formed fascicles; brightly eosinophilic cytoplasm	–	+	+ (most)	–	+ (10–15%)	Variable karyotype; no consistent pattern of gene involvement
Gastrointestinal-tract schwannoma	Spindle cell; short intersecting fascicles; lymphocytic infiltrate; variable palisading	–	–	–	+	+	Deletion of 22q; *NF2* inactivation in about 50%
Desmoid fibromatosis	Spindle cell; long fascicles; collagenous stroma; palely eosinophilic cytoplasm	–*	+	+ (rare cells)	+ (rare cells)	+ few	Deletion of 5q; trisomies 8 and 20; *APC* mutation in about 50%

*Most, but not all, investigators report that the vast majority of fibromatoses are KIT-negative. SMA, smooth-muscle actin.

Cell of origin

Kindblom and colleagues suggested in 1998 that GISTs originate from stem cells that differentiate towards the interstitial cells of Cajal (ICCs), and that GISTs should be called gastrointestinal pacemaker-cell tumours.24 ICCs arisefrom precursor mesenchymal cells and are the pacemaker cells that bring about autonomous movement of the gastrointestinal tract.27 They intercalate between nerve fibres and muscle cells and can be seen in the adult intestine in and around the myenteric plexus.2 Both ICCs and GISTs express KIT protein, have similar ultrastructural features, and express the embryonic form of the heavy chain of smoothmuscle myosin;24,28 all these features support a common histogenesis.

The precursor-cell hypothesis could also explain why KIT-expressing mesenchymal tumours, with similar histology to GISTs, can arise outside the gastrointestinal tract in the omentum, mesentery, and retroperitoneum.29,30 ICC-like cells have also been identified in the omentum.31 The precursor-cell hypothesis could also account for the coexpression of KIT, SMA, and even desmin in some GISTs. In studies of immunohistochemistry and confocal microscopy of normal intestinal muscle, CD34 immunopositivity has been found in fibroblast-like cells located near the ICCs. Conversely, ICCs did not stain for CD34 in these studies, and no cells with concomitant CD34 and KIT positivity were found. These findings also support the hypothesis that GISTs originate from a more primitive precursor cell.32

Diagnosis

The clinical prediagnostic investigation of GISTs is similar to that of other gastrointestinal malignant disorders. A doublecontrast series of radiographs may show a characteristic smooth-lined filling defect with clearly demarcated borders. On endoscopic examination, there may be a smooth protrusion of the bowel wall, lined with mucosa, which can also

show signs of bleeding and ulceration.14 Endoscopic ultrasonography may show a hypoechoic mass that is contiguous with the muscularis propria of the normal gut wall. In one ultrasonography study, presence of malignant GIST was suggested by the presence of a large (>4 cm) tumour with irregular extraluminal border, echogenic foci, and cystic spaces.33 Computed tomography (CT) and magnetic resonance imaging (MRI) are essential in assessment of primary tumour extension and the presence of metastases.

Most GISTs are submucosal and grow endophytically, which decreases the likelihood that a tissue diagnosis can be obtained preoperatively. Only about 50% of patients assessed by endoscopy are given a histological diagnosis preoperatively. Percutaneous fine-needle aspiration has been suggested as an initial diagnostic technique, if feasible, but it is not universally recommended because intra-abdominal tumour spillage is possible.14,34 Most oesophageal stromal tumours are benign,22 and endoscopic biopsy, for cases in which the mucosa surrounding the tumour is intact, is controversial because of the potential high risk of intraoperative oesophageal perforation.14,35

During laparotomy, all resected margins should be carefully oriented and examined, and biopsy samples from grossly different areas of the excised tumour should be evaluated pathologically. Solid and firm areas should be included, as well as necrotic and haemorrhagic parts of the tumour. A minimum of one tissue section per centimetre of tumour diameter has been recommended for microscopic assessment of fixed tissue.14 KIT immunostaining should be done with appropriate positive and negative control samples to avoid falsely positive and negative results. Mast cells and ICCs stain strongly positively for KIT and may be used as positive internal controls.

Immunohistochemical staining for KIT (CD117), CD34, desmin, SMA, and S100 should be carried out. Mutation analysis of *KIT* is not considered mandatory in routine diagnostics, but this technique is currently being evaluated to see whether it might provide predictive information about the likelihood of a response to imatinib mesylate therapy. Additionally, a *KIT* mutation gives further support to the diagnosis of GIST.

Prognostic features

Assessment of the malignant potential of a primary GIST lesion is difficult in many cases, and even small GISTs (less than 2 cm in diameter) have uncertain malignant potential.3 The criteria used to predict biological behaviour also vary significantly with tumour location, for example, smoothmuscle tumours arising from the small bowel, colon, rectum, omentum, or mesentery are generally associated with a less favourable outcome than those arising in the stomach.12 Most oesophageal and colonic GISTs are malignant, whereas in the case of gastric GISTs, more indolent tumours seem to outnumber overtly malignant ones. However, with long follow-up their outcome may not differ greatly. Almost all incidental small (<1 cm) GISTs are clinically benign, whereas tumours larger than about 5 cm in diameter are generally malignant. Nonetheless, no cut-off diameter predicts subsequent malignant behaviour with certainty, and the optimum cut-off size may vary for different sites.12,13,15,36

The mitotic rate is one of the more reliable single factors in differentiating between GISTs of varying malignant potential. In general,

most tumours with 0–1 mitoses per 10–50 high-power fields (HPFs) will not give rise to metastases, those with over five mitoses per 50 HPF are considered as malignant, and tumours with over 20–50 mitoses per 50 HPF are classed as high-grade malignant.2,13–15.

However, a low mitotic count does not rule out malignancywith certainty, and vice versa, and the mitotic count is of limited value especially in assessment of the malignant potential of small-bowel GISTs.12 Other factors suggested to be associated with an adverse outcome include: incomplete surgical resection and tumour rupture at surgery;6 infiltration of tumour to the neighbouring structures; location of the primary tumour in the intestine;22 presence of coagulative tumour necrosis, high cellularity, and pronounced pleiomorphism; a high S-phase fraction and DNA aneuploidy in flow cytometry or image cytometry; a high Ki-67 score; proliferating-cell nuclear-antigen expression; and presence of telomerase activity. In some studies the presence of *KIT* mutation has also been implicated.12,18. However, many of these features are predictive of outcome only in statistical analysis of large series of cases, and their usefulness on an individual-case basis is limited.

Altogether, there are many borderline malignant tumours within the spectrum of GISTs, ranging from indolent tumours to clearly malignant cancers. All GISTs should be considered as having some low malignant potential, and they should be described in terms of risk assessment, rather than using distinct benign and malignant categories.3 The only certain indication of malignancy is tumour spread beyond the organ of origin at the time of diagnosis.13 However, most primary GISTs larger than 5 cm in diameter and with a mitotic count higher than five per 50 HPF are likely to behave in a malignant way. Similarly, GISTs larger than 10 cm in diameter have a high risk of aggressive behaviour whatever the mitotic count, and GISTs of any size with a high mitotic count (more than ten per 50 HPF) are also deemed to be high-risk tumours.3

Outcome

Before about the year 2000, studies of GISTs included tumours that would not presently be classified as GISTs, and data are therefore contaminated by these cases. However, since GISTs constitute the majority of gastrointestinal sarcomas, the survival data from these

studies probably largely reflect the experience of patients with true GISTs. Most recurrences take place within 5 years of the primary diagnosis,12 but in the slowly proliferating subset of GISTs, metastases can appear more than 10 years after the primary diagnosis.

Outcome depends on the histopathological and clinical features. The reported overall or disease-specific 5-year survival is 28–60% among patients with malignant GIST; the median disease-specific survival is about 5 years for primary disease, and 10–20 months in recurrent or metastatic disease.1,6,16,18. According to one study, patients who have been diagnosed with GISTs with one to five mitoses per 50 HPF have median survival of 98 months, whereas those with GISTs with more than ten mitoses per ten HPFs have median survival of 25 months.42 In another study, patients who had undergone curative surgery had 8-year disease-free survival of 80% when fewer than ten mitoses were present per 50 HPF compared with an 18- month median survival in patients with more high-grade lesions.

Molecular biology

The fundamental pathogenetic feature of the most common malignant phenotype of GIST seems to be activation of the KIT signalling pathway. KIT is a transmembrane tyrosine kinase encoded by the *KIT* proto-oncogene located on chromosome 4q11-q12.45 It is the cellular homologue of the oncogene *v-kit* of the Hardy-Zuckerman feline sarcoma virus. The natural ligand of KIT is SCF (also known as the mast-cell growth factor, Steel factor, or the KIT ligand). Unbound KIT protein is an enzymatically inactive monomer spanning the plasma membrane. Soluble SCF is predominantly a bivalent dimer, which causes homodimerisation of KIT by binding to the extracellular domain. This action results in activation of KIT via autophosphorylation of intracellular tyrosine residues.15.

Autophosphorylation creates docking sites for signal transduction molecules. Activated KIT then functions as a tyrosine kinase, transferring phosphate groups from ATP to the tyrosine residues of target proteins, which become activated in turn. The activation signalling cascade to the nucleus involves several proteins including MAP kinase,

PI3 kinases, STAT5, RAS, and JAK2,15,48-51 which have been implicated in KIT-induced mitogenesis and differentiation.

Structurally, KIT tyrosine kinase is a type III tyrosine kinase receptor, similar to receptors of macrophage colonystimulating factor, platelet-derived growth factor, and the haemopoietic growth factor FLT3 ligand, each possessing five immunoglobulin-like extracellular repeats and a tyrosine kinase domain split into two by an insert sequence of variable length (figure 2). In addition to ICCs, KIT is normally expressed in mast cells, melanocytes, Leydig cells, spermatogonia, spermatids, haemopoietic stem cells, cutaneous basal cells, and breast epithelial cells,25,52 and it has important roles in haemopoiesis, melanogenesis, gametogenesis, and the development of mast cells and ICCs.27.

Almost all GISTs have constitutive activation (phosphorylation) of the KIT protein, and most have inframe mutations that preserve the expression of KIT. Stable transfection of mutated *KIT* cDNAs into murine lymphoid cells causes malignant transformation.5. Unlike the normal KIT protein, mutated KIT may not require SCF for dimerisation or autophosphorylation. This ligandindependent activation causes a shift

in the balance between cell survival and proliferation away from apoptosis. The frequency of *KIT* mutations in GISTs varies in different studies, according to the tumour type, and probably because of differences in the methods used to analyse the tumours. Up to 80–90% of metastatic GISTs have been reported to have mutated *KIT*, and in some studies *KIT* mutations have been found in benign, borderline, and malignant GISTs at about equal frequency. Corless and co-workers found identical *KIT* mutations to those previously found in larger GISTs, in 11 (85%) of 13 of morphologically benign GISTs that were found incidentally at autopsy, endoscopy, or laparotomy, by use of a sensitive mutation-detection method (denaturing high-performance liquid chromatography). These small GISTs were all immunohistochemically positive for KIT, and ranged in size from 4 mm to 10 mm. If confirmed, these findings suggest that *KIT* mutations are acquired very early in the development of most GISTs and that *KIT* mutations alone may be of limited prognostic importance. *KIT* mutations have not been found in leiomyomas and leiomyosarcomas.

Family members with germline mutations of *KIT* have indolent and malignant GISTs from a young age and have the same mutation in

the germline, indolent GISTs, and malignant GISTs, which also suggests that *KIT* mutation is an early oncogenic event.15 *KIT* has 21 exons, and in sporadic GISTs the *KIT* mutations have been found in exon 11, encoding an intracellular juxtamembrane region of the receptor, in 50–77% of cases. Exon-9 mutations, encoding a region located in the extracellular domain, are found in 3–18% of GISTs, and a few GISTs have mutations in exons 13 and 17, encoding the intracellular part of the receptor. Mutation of *NF2* gene has also been described in GISTs. This finding is consistent with high incidence of GISTs in patients with neurofibromatosis. Loss of chromosome 1p or complete or partial loss of chromosomes 14 and 22 occurs in 50% or more of GISTs and may be involved in GIST pathogenesis and progression. However, such changes may be secondary, and activating mutations of *KIT* seem to have a central role in GIST pathogenesis.44 This idea is supported by a recent study that used 13, 826-element, cDNA microarrays to analyse gene-expression patterns of *KIT*-mutation-positive GISTs.

In this study the expression profiles of GISTs were remarkably uniform with the *KIT* gene ranking the highest on the discriminator list and being highly expressed in every tumour studied. Therefore, mutated

KIT is an excellent molecular target for therapeutic interventions with KIT-selective tyrosine-kinase inhibitors.

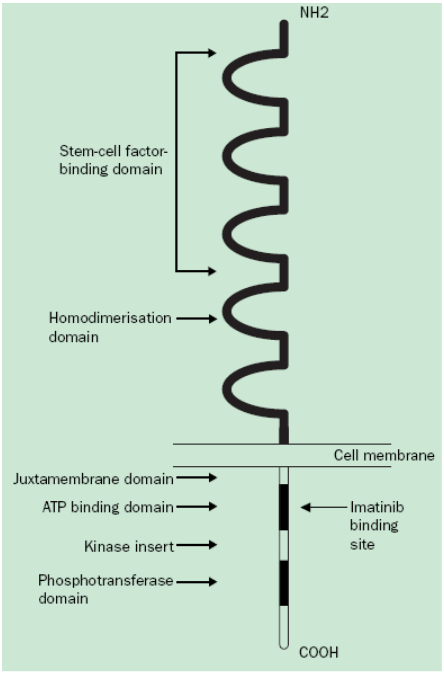


Figure 2. The structure of the KIT receptor tyrosine kinase.

Surgery

Surgery remains the standard initial treatment for nonmetastatic GISTs. Careful pathological assessment should be done to differentiate GISTs from carcinomas and lymphomas. As with other soft-tissue sarcomas, a true capsule does not exist, and the tumour should be removed en-bloc with its pseudocapsule and, if possible, an adjacent margin of normal soft tissue or bowel. In cases where contiguous organs

are involved, en-bloc resection has been recommended wherever feasible.14 Since this process may require total gastrectomy in the case of gastric GISTs, pancreaticoduodenectomy for a duodenal GIST, or an abdominoperineal resection for tumours involving the rectum, associated morbidity can be substantial. In such cases, preoperative, neoadjuvant treatment with imatinib mesylate may eventually be considered, but at present no data are available to support this practice. A trial evaluating this treatment option is in progress in the USA.

In several studies, patients who had complete tumour resection had better overall survival than those who had less radical surgery.16. Although this difference may partly reflect aggressive biological features of GISTs that cannot be totally removed, an effort should be made to obtain histologically tumour-free tissue margins. The optimum width of the tumour-free margin has not been defined. Tumour rupture, spontaneously or during surgery, may be associated with an increased risk of development of peritoneal implants and should be avoided. Regional lymph-node resection is of unproven value, and extensive lymphadenectomy is not recommended.14.

There are few data about the usefulness of resection of recurrent disease or intra-abdominal metastases. In some studies, tumour-specific mortality and overall survival have not differed significantly between patients who underwent complete resection of recurrent disease and those who had partial resection or biopsy alone.21 However, there is evidence that metastasectomy may improve survival in selected patients. Patients with well or moderately differentiated GIST, a disease-free interval between the diagnosis and detection of metastases of longer than 12 months, and isolated resectable liver metastases are more likely to benefit from metastasectomy than patients who have rapidly progressing or widespread GIST. Many patients who have a bleeding tumour or tumour-related bowel or biliary-tract obstruction achieve efficient palliation with surgery.

However, since imatinib mesylate was developed as a therapeutic alternative, surgery for metastatic GIST has largely been replaced by drug therapy, and primary surgery for metastatic GIST should probably be attempted only in patients who have bleeding or obstructive disease. The question of whether surgical resection should be done to remove residual masses, after imatinib mesylate therapy, is unanswered and

requires further research. We suspect that this approach would be a very reasonable option for patients with low-volume metastatic disease.

Radiotherapy

The impact of radiotherapy on outcome is unknown. Many visceral sarcomas are not readily amenable to radiotherapy because of organ motility, and postoperatively contaminated bowel loops may relocate to remote sites. The large target volumes needed and the limited radiation tolerance of the intra-abdominal organs limit the usefulness of radiotherapy. Fixed lesions on the abdominal wall or adjacent organs have been treated with postoperative radiotherapy, but recurrences both within and outside the radiation field have been frequent.16 At present, radiotherapy is not a standard postoperative therapy for GIST, and in most cases should be reserved for limited palliative settings or for research of new strategies.

Chemotherapy

Attempts to treat malignant GISTs with systemic chemotherapy have been almost universally unsuccessful. In one study, only 3 of 43

(7%) patients with gastrointestinal soft-tissue sarcomas (most tumours probably GISTs) responded to a combination of doxorubicin and dacarbazine, whereas 22% of patients with uterine leiomyosarcomas and 21% of patients with leiomyosarcomas of other sites responded to this combination (p=0·05), suggesting relative chemoresistance of gastrointestinal soft-tissue sarcomas. Similar results have been obtained in other studies. Only 1 of 21 (5%) patients with GISTs treated with combination chemotherapy consisting of dacarbazine, mitomycin c, doxorubicin, cisplatinum, and sargramostine showed a response, and no gastrointestinal soft-tissue sarcomas responded to a combination of etoposide and ifosfamide.

The unresponsiveness of GISTs to drugs commonly used in the treatment of soft-tissue sarcomas may be explained partly by the frequent expression of P-glycoprotein and multidrug resistance protein 1 (MDR1) in GISTs. study, 38·4% of GISTs expressed P-glycoprotein and 35·4% expressed MDR1 protein, whereas only 13·4% and 13·3% of gastrointestinal leiomyosarcomas, respectively, stained positively for these proteins.19In onestudy, 38·4% of GISTs expressed P-glycoprotein and 35·4% expressed MDR1 protein, whereas only 13·4% and 13·3% of

gastrointestinal leiomyosarcomas, respectively, stained positively for these proteins.19

Imatinib mesylate

Imatinib mesylate is a competitive inhibitor of certain tyrosine kinases including the intracellular kinases ABL and BCR-ABL fusion protein present in some leukaemias, KIT, and the platelet-derived growth factor receptors. Imatinib mesylate inhibits these tyrosine kinases at submicromolar concentrations, but has little or no effect on many other tyrosine or serine/threonine kinases. It is a small multiringed molecule, which competes with ATP for its kinase-binding site, and prevents the kinase from transferring phosphate from ATP to tyrosine residues of the substrates. This action inhibits downstream signalling from the kinase, which switches the balance towards apoptosis. Imatinib mesylate is very well absorbed after oral administration and is available as capsule formulations. It is metabolised mainly in the liver by the P450 isoenzyme CYP3A4, and the metabolites are mostly excreted via the bile into the stools. The half-life in the circulation is about 20 h, which is compatible with oncedaily administration. Preclinical studies suggest that maintenance of imatinib mesylate serum concentrations above 1

_mol/L are needed for optimum therapeutic effects, and such concentrations are obtained in most patients with daily doses of 300 mg or greater.

Effectiveness of imatinib mesylate in advanced GIST

Based on two studies, imatinib mesylate is the first effective drug in the treatment of metastatic GIST. The US–Finland study reported a response rate of 54% among 147 patients with inoperable or metastatic GIST treated with a daily dose of 400 mg or 600 mg with follow-up of at least 6 months. In addition, 28% had minor response or stable disease, and only 14% showed primary resistance to the drug. Similar results were reported from a trial by the European Organization for Research and Treatment of Cancer, in which 36 patients with advanced GISTs were treated with a daily dose of 400–1000 mg. 53% had confirmed partial responses and 17% had as yet unconfirmed partial responses or more than20% regression, and only 11% of patients had progression. In these studies, about 90% of patients with symptoms had marked relief of them. In both studies, inclusion required histology compatible with GIST and KIT expression verified by immunohistochemistry.

Response to imatinib mesylate can occur rapidly, even in patients with a large tumour burden but regression can also be slow, particularly after an initial rapid phase of tumour regression. The median time to response is about 13 weeks. Interestingly, tumour lysis syndrome has not been described in these patients, even though hydration or allopurinol have not been routinely administered alongside imatinib mesylate. Liver lesions commonly acquire a cyst-like appearance after the start of treatment, and may thus seem better delineated on magnetic resonance (MR) or computed tomography (CT) images they should not be confused with new lesions or progressive disease. Histologically, the cyst-like lesions consist of hyaline degeneration, but a few remaining KIT-positive cells could represent dormant or slowly proliferating GIST cells and may persist for several months in these lesions. The malignant potential of the few persistent intralesional KIT-positive cells is not currently known.

In addition to CT and MRI, fluorodeoxyglucose (labeled with fluorine-18) positron emission tomography (FDG-PET) may be a useful imaging technique to assess response to imatinib mesylate. Glucose uptake of GIST lesions decreases within a few hours to a few days after

the start of imatinib mesylate treatment, which can be verified by FDG-PET. The technique could be helpful in problematic cases to make a distinction between intratumoral bleeding and disease progression. The PET scan responses can also predict subsequent tumour volume reductions found on CT or MRI. In one study, patients with an exon-11 *KIT* mutation had a significantly higher rate of response to imatinib mesylate therapy (72%) than patients whose tumour had an exon-9 mutation (32%) or no detectable mutation (12%), and the time to treatment failure was also longer in patients with an exon-11 mutation.

The optimum dose of imatinib mesylate in the treatment of GISTs is not yet known and is being studied in current randomised phase III trials. Toxicity increases with increasing dose, and in one study, 800 mg was the maximum tolerated dose when taken for 8 weeks. No statistical difference in the response rate was found in another study of 400 mg and 600 mg daily doses, although these data are underpowered and the results of ongoing large-scale studies are needed before conclusions are reached.

Data from studies on chronic myeloid leukaemia suggest that doses less than 300 mg may be too small for the competitive inhibition of the BCR-ABL kinase and should be avoided in clinical practice. Overall, present data suggest that a daily dose of400 mg or higher should be used in the treatment of patients with metastatic or unresectable GIST.

The optimum treatment duration remains unknown, but in metastatic disease administration of imatinib mesylate for several months to a few years is likely to be needed. In the US–Finland study, objective major responses were durable and generally continuing at follow-up of at least 9 months, with no median duration of responses yet evident. So far, responses lasting up to 24 months have been observed but secondary resistance to imatinib mesylate has been seen in some patients who initially responded to the drug. The proportion of patients who will relapse after a response is not currently known.

Tolerability and safety of imatinib mesylate

Tolerability has been acceptable with daily doses of 800 mg or less. The most common adverse effects include periorbital and leg oedema transient nausea associated with drug ingestion, muscle cramps, diarrhoea, headache, dermatitis, fatigue, anaemia, and neutropenia. Most of these side-effects are mild to moderate in severity, and grade 3–4 toxic effects occur in less than 30% of patients at a dose of 400–600 mg per day.A few patients (5%) have had a tumour-associated bleeding either into the abdominal cavity or into the bowel, and patients who develop symptoms that suggest acute bleeding may need emergency care.

Most adverse effects resolve within a few days to weeks after cessation of treatment, and most patients can continue at a lower dose. Imatinib-mesylate-associated oedema can be treated with diuretics. Drug-ingestion-related nausea rarely requires antinausea medication, although some patients are given a divided dose.

The muscle cramps typically occur in the fingers and legs, and are transient, most requiring no specific therapy. Interactions with other drugs metabolized by CYP3A4 are possible. In particular, concomitant use of paracetamol or warfarin is not recommended

Future directions and unresolved questions

Imatinib mesylate is a major breakthrough in the treatment of advanced GISTs and is the first effective systemic therapy for this disease. However, several important questions remain unanswered: the required duration of imatinib mesylate therapy; the proportion (if any) of the patients with metastatic disease who will achieve long-term disease control, and whether treatment results can be improved with combination therapies.

In the treatment of BCR-ABLexpressing leukaemia, resistance to imatinib mesylate is commonly associated with reactivation of BCR-ABL signalling due either to a secondary mutation resulting in substitution of threonine with isoleucine at a critical binding site of the drug or to progressive *BCR-ABL* gene amplification.80 Little is known

about the resistance mechanisms of imatinib mesylate in the treatment of GISTs, but these are currently being studied.

An important strategy, which is likely to affect cure rates, is whether adjuvant therapy will benefit patients after macroscopically complete removal of malignant GIST, or after excision of GIST with intra-abdominal metastases amenable to surgical removal. Many such patients will develop inoperable systemic metastases within a few years of primary surgery, and adjuvant therapy with imatinib mesylate for subclinical metastatic disease might lead to a better cure rate than treatment for overtly metastatic disease. In the absence of data from clinical trials, there is no objective basis for recommending adjuvant use of imatinib mesylate, but the potency of the drug in metastatic disease certainly justifies the rapid application and study in the adjuvant setting. Similarly, the role of neoadjuvant imatinib mesylate needs to be studied especially in cases where extensive surgery resulting in loss of organ function is the only other option. Combination therapies with conventional chemotherapeutic drugs and other signal transduction inhibitors also need to be investigated.

The molecular targeting of the critical pathogenetic mechanism underlying GIST has given patients new hope, and has provided physicians with a highly effective and well tolerated therapeutic option for a disease for which no systemic therapy existed previously.

RESULTS

Thirty one patients with GIST treated at our institute were included in the study. The median age was 55 years (14-70 years). From the 31 patients were 8 (25.8%) females and 23 (74.2%) males. Among these, 24 patients has first time surgery for a GIST at our institute and 7 patients were presented with advanced metastatic disease which were deemed inoperable. All the 7 patients were started on Imatinib.

Out of the 24 operated patients, 2 patients presented with metastasis, primary alone was resected. Detailed tumour localizations are shown in Table 1. Applying the Fletcher classification, 4 patient had low risk, 4 had intermediate risk and 16 patient had high risk. This reflects that the majority of the patients had an intermediate or high risk of an aggressive behaviour of their GIST. Regarding tumour size 5/24 of all tumours were smaller and 19/24 larger than 5 cm.

Table 1 Tumour characteristics

	Stomach	Small intestine	Rectum	Total
Tumuor size				
< 5cm	2	2	1	5
5-10cm	7	1	1	9
10-15cm	2	-	-	2
15-20cm	3	3	1	7
>20cm	1	-	-	1
Total (n)	15	6	3	24
Mitosis				
<5/50 hpf	7	2	-	9
>5/50 hpf	8	4	3	15
Fletcher classification				
Very low risk	-	-	-	0
Low risk	2	2	-	4
Intermediate	3	-	1	4
High	10	4	2	16
Necrosis +	12	2	1	15
Type of resection				
Segmental /TG	10	4	-	14
APR			2	2
Multi organ	5	2	1	8
Status				
Disease free	12	1	1	14
Recurrent disease	3	5	2	10

Surgical procedures

The surgical procedures carried out in this study varied from wedge resection of the tumour to complex multivisceral surgery. Multiorgan resection were performed in 8/24 patients. All the surgical procedures are summarized in Table 2. Complete tumour resection (R0) was achieved in 22/40 patients, whereas 2/24 patients had incomplete tumour resection (R2). One patient who underwent APR had positive iliac nodes (2/5), which is a rare feature of GIST.

Table 2 Surgical procedures

Resection of stomach	
Wedge resection	13
Total gastrectomy	2
Small bowel resection	
Segmental resection	5
Whipple procedure	1
Rectal surgery	
Abdominoperineal resection	2
APR+ prostatectomy	1
Splenectomy	5
Distal pancreatectomy	2
Partial liver resection	1
Partial cystectomy	1
Tumour spill	4
R0 resection	22
R1 resection	-
R2 resection	2

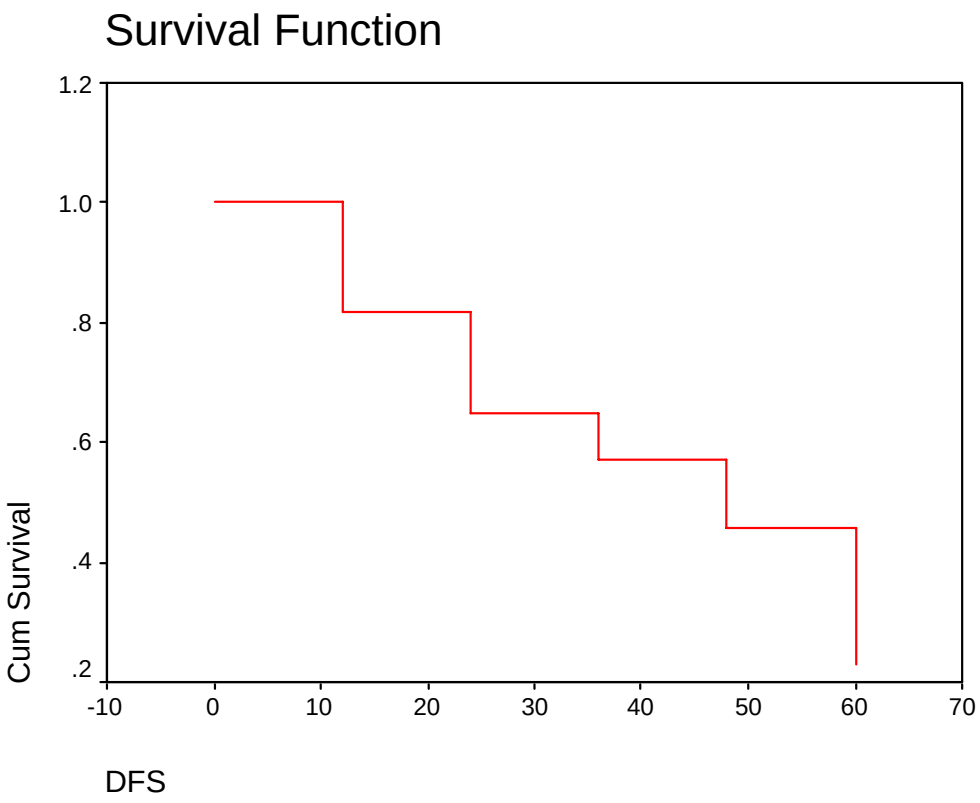
Complications

Post-operative complications were observed in 3/24 patients. There was no post-operative mortality (until day 30). One patient had urinary fistula following APR and another patient had faecal fistula following multiorgan resection. Both these patient had incomplete tumour resection (R2). The third patient had persistent post-operative fever, managed appropriately.

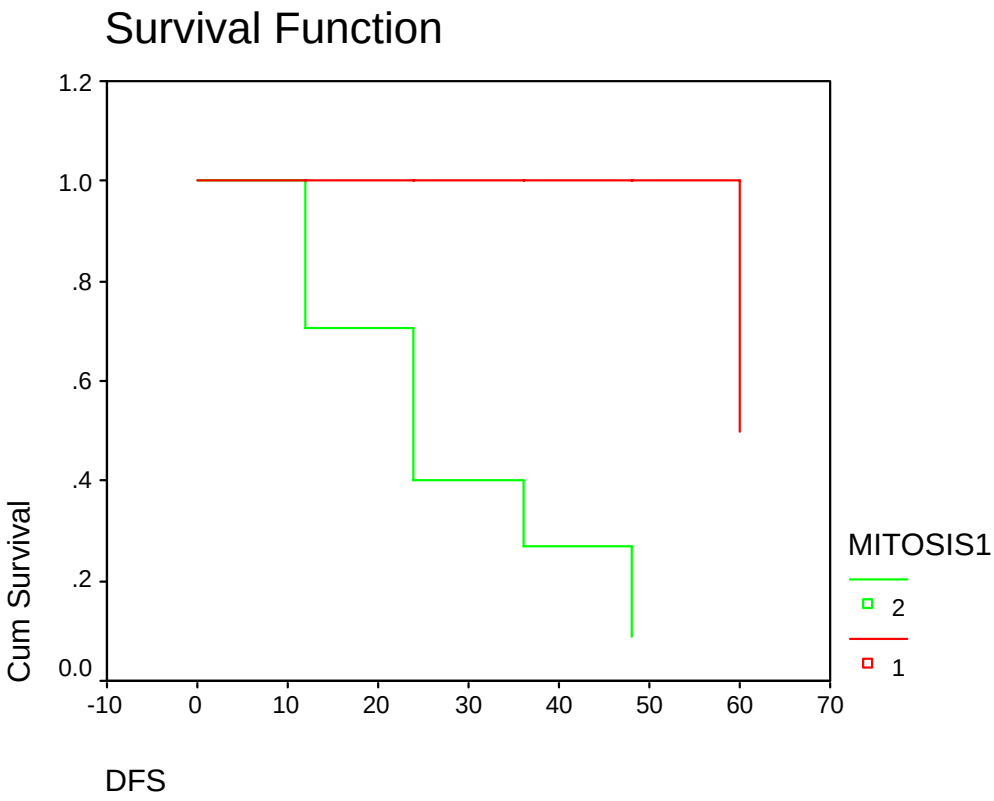
Survival and tumour recurrence

The median disease free survival time was 43.6 months with a median followup of 13 months (6- 88 months). At 5 years the probability of disease free survival was 45.8%. 10 out of 24 patients developed recurrence on follow up. In five patients liver alone was the site of metastasis. One patient developed metastases in liver and peritoneum. One developed recurrence in the small bowel, one on the dome of the urinary bladder and one patient developed pelvic recurrence. One patient developed lung metastasis. Among the 10 patients who developed recurrent disease three underwent secondary surgery, resulting in R0

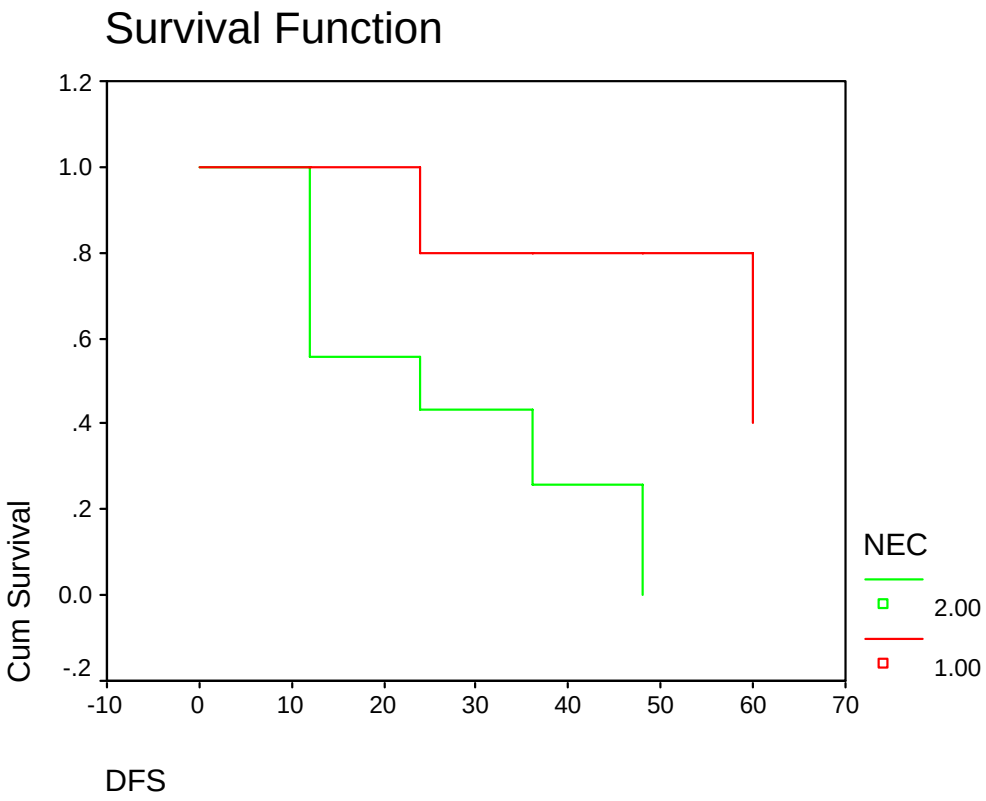
resection. The secondary resections were, one liver metastasectomy, one segmental resection of jejunum and one partial cystectomy. Among the recurrences the primary sites of GIST were small intestine in 5 patients, stomach in 3 patients and rectum in 2 patients. All the 10 patients were started on Imatinib. Out of this 10 patients, one patient died after 4 months of primary surgery. Remaining 9 patients are alive with disease till date.



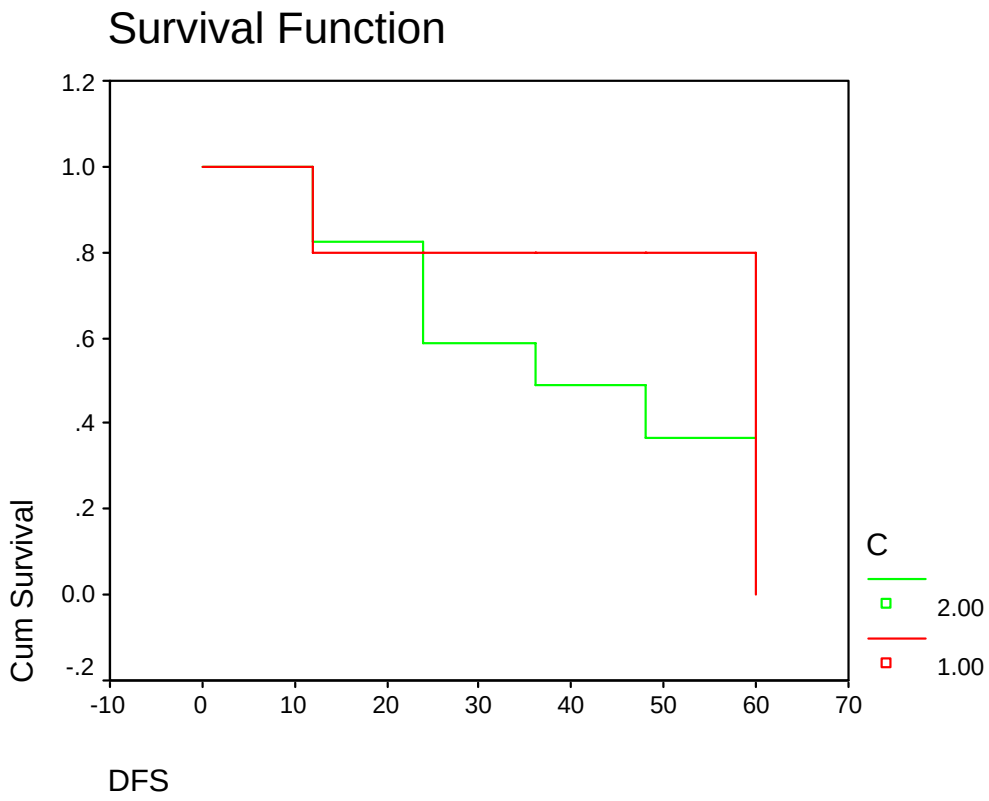
The probability of DFS at 5 years is 100% for tumours with mitotic count <5/50 hpf and 26.8% at 3 years for >5/50 hpf (p=0.006).



The probability of DFS at 5years for tumours without necrosis is 80% and 26% at 3 years for tumours with necrosis (p=0.0046).



Tumour size >5cm was also found to be a poor prognostic factor although it did not reach statistical significance. The probability of DFS at 5 years for tumours <5 cm is 80% and 36.8% at 5 years for tumours >5 cm (p=0.54).



Positive margin is a strong prognostic factor for recurrence. Out of the 24 operated patients, 2 patients had positive margins and both of them recurred. The primary tumour site was also found to be a strong prognostic factor for recurrence. Out of the 6 patients with primary GIST of small intestine 5 recurred (one patient had R2 resection). Among the 6 patients with rectal primary 2 have recurred (one had R2resection). Out of the 15 patients with primary GIST of stomach, only 3 have recurred. The extend of surgery and tumour spill were not found to be of any prognostic significance. Patient characteristics like age and sex showed no prognostic value for the development of a recurrent disease.

DISCUSSION

Most studies of the clinicopathological entity referred to as GIST before the year 2000, are likely to include a group of patients with true GISTs as well as other histological subtypes of spindle-cell sarcoma such as leiomyosarcoma.

Our study included patients on the basis of c-kit positivity.

GISTs occur in both sexes at a similar frequency, but some data show male predominance.2,6 The median age at diagnosis is about 60.

Our male: female ratio is 4:1, and the median age is 55 years.

GISTs are most commonly found in the stomach (40–70%), but they can occur in all other parts of the gastrointestinal tract. About 20–40% of GISTs arise from the small intestine, and 5–15% from the colon and rectum.1,2,12,13 GISTs can also be found in the oesophagus (<5%), omentum (<5%), mesentery, or retroperitoneum.2,3,12.

In our series also the commonest site is stomach, constituting 62.5%. 40–80% of GISTs recur despite histopathologically complete tumour resection. The most common sites of metastases are the peritoneum and the liver,1,6 whereas regional lymph-node metastases are extremely rare.1,20.

In our study also 41.66% developed recurrence. Commonest site of recurrence is liver. One patient with GIST of rectum had lymph node metastasis.

Despite the great success of Imatinib in the treatment of metastatic GIST, primary surgery remains the cornerstone in the treatment of localized and resectable GIST. Recurrent disease is still a great problem. Therefore primary risk adapted surgery is the most important factor to avoid an early tumour recurrence and it is important to identify patients who may be candidates to receive an adjuvant treatment after complete tumour resection.

The criteria used to predict biological behaviour vary significantly with tumour location, for example, smooth muscle tumours arising from the small bowel, colon, rectum, omentum, or mesentery are generally associated with a less favourable outcome than those arising in the stomach.12

Our series supports the theory that GIST arising in sites other than stomach has a more aggressive behaviour. In our series Out of the 6 patients with primary GIST of small intestine 5 recurred (one patient had R2 resection). Among the 6 patients with rectal primary 2 have recurred (one had R2resection). Out of the 15 patients with primary GIST of stomach, only 3 have recurred.

As per our results primary GIST of small intestine is the most aggressive and GIST of stomach is the least aggressive, similar to results shown by most of the studies. 6,12.

The mitotic rate is one of the more reliable single factors in differentiating between GISTs of varying malignant potential. In general, most tumours with 0–1 mitoses per 10–50 high-power fields (HPFs) will not give rise to metastases, those with over five mitoses per 50 HPF are considered as malignant, and tumours with over 20–50 mitoses per 50 HPF are classed as high-grade malignant.2,13–15. However, a low mitotic count does not rule out malignancy with certainty, and vice versa, and the mitotic count is of limited value especially in assessment of the malignant potential of small-bowel GISTs.12

Our results showed strong correlation of mitotic count with tumour recurrence, which reached statistical significance. The probability of DFS at 5 years is 100% for tumours with mitotic count <5/50 hpf and 26.8% at 3 years for >5/50 hpf (p=0.006).

Other factors suggested to be associated with an adverse outcome include: incomplete surgical resection and tumour rupture at surgery;6 infiltration of tumour to the neighbouring structures; multiorgan resection, location of the primary tumour in the intestine;22 presence of coagulative tumour necrosis, high cellularity, and pronounced pleiomorphism; a high S-phase fraction and DNA aneuploidy in flow cytometry or image cytometry; a high Ki-67 score; proliferating-cell nuclear-antigen expression; and presence of telomerase activity. 12,18.

In our series also incomplete resection is a strong predictive factor of recurrence. Out of the 24 operated patients, 2 patients had positive margins and both of them recurred.

Presence of necrosis in the tumour showed strong correlation with tumour recurrence, which reached statistical significance. The probability of DFS at 5years for tumours without necrosis is 80% and 26% at 3 years for tumours with necrosis (p=0.0046).

Tumour spill alone, with complete resection of the tumour did not show a prognostic value for recurrence in our study. Similarly extend of surgery did not influence DFS in our series.

Currently adjuvant therapy trials are being conducted to evaluate the benefit of Imatinib in those GIST patients who have a substantial risk of relapse after complete surgery. Furthermore the administration of Imatinib in patients with advanced GIST preoperatively to avoid multivisceral surgery is still an individual decision as data from a controlled trial are still missing.

CONCLUSION

Primary surgery remains the cornerstone in the treatment of localized and resectable GIST. Recurrent disease is still a great problem. Summarizing our results with the influence of high mitotic count, presence of tumour necrosis, large tumour size, incomplete surgical resection and primary site other than stomach on the DFS, which is reflected by a high rate of recurrences, we support the concept that all patients with these high risk features need to be assessed for adjuvant treatment with Imatinib.

Considering the 100% chance of recurrence with an incomplete resection, neoadjuvant treatment with Imatinib followed by surgical resection and continuing of Imatinib administration is a reasonable option. This combination strategy may provide survival benefit. But it remains to be determined whether this multimodal approach may exceed the benefits associated with Imatinib monotherapy.

REFERENCES

1. Beghini A, Tibiletti M, Roversi G, et al. Germline mutation in the juxtamembrane domain of the KIT gene in a family with gastrointestinal stromal tumors and urticaria pigmentosa. *Cancer* 2001; **92:** 657–62.

2. Bonavina L, Segalin A, Rosati R, et al. Surgical therapy of esophageal leiomyoma. *J Am Coll Surg* 1995; **181:** 257–62.

3. Carney JA. Gastric stromal sarcoma, pulmonary chondroma, and extra-adrenal paraganglioma (Carney triad): natural history, adrenocortical component, and possible familial occurrence. *Mayo Clin Proc* 1999; **74:** 543–52.

4. Catena F, Pasqualine E, Campione O. Gastrointestinal stromal tumors: experience of an emergency surgery department. *Dig Surg* 2000; **17:** 503–07.

5. Chak A, Canto MI, Rosch T, et al. Endosonographic differentiation of benign and malignant stromal cell tumors. *Gastrointest Endosc* 1997; **45:** 468–73.

6. Clary BM, DeMatteo RP, Lewis JJ, et al. Gastrointestinal stromal tumors and leiomyosarcoma of the abdomen and retroperitoneum: a clinical comparison. *Ann Surg Oncol* 2001; **8:** 290–99.

7. Crosby JA, Catton CN, Davis A, et al. Malignant gastrointestinal stromal tumors of the small intestine: a review of 50 cases from a prospective database. *Ann Surg Oncol* 2001; **8:** 50–59.

8. DeMatteo RP, Lewis JJ, Leung D, et al**.** Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. *Ann Surg* 2000; **231:** 51–58.

9. Emory TS, Sobin LH, Lukes L, et al. Prognosis of gastrointestinal smooth-muscle (stromal) tumors. *Am J Surg Pathol* 1999; **23:** 82–87.

10. Fletcher CDM, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum Pathol* 2002; **33:** 459–65.

11. Fong Y, Coit DG, Woodruff JM, et al. Lymph node metastasis from soft tissue sarcoma in adults: analysis of data from a prospective database of 1772 sarcoma patients. *Ann Surg* 1993; **217:** 72–77.

12. Heinrich MC, Blanke CD, Druker BJ, et al. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002; **20:** 1692–703. 26 Hornick JL, Fletcher CDM. Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. *Am J Clin Pathol* 2002; **117:** 188–93.

13. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of *c-KIT* in human gastrointestinal stromal tumors. *Science* 1998; **279:** 577–80.

14. Ishida T, Wada I, Horiuchi H, et al. Multiple small intestinal stromal tumors with skeinoid fibers in association with neurofibromatosis 1 (von Reclinghausen's disease). *Pathol Int* 1996; **46:** 689–95.

15. Kindblom LG, Remotti HE, Aldenborg F, et al. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the intestinal cells of Cajal. *Am J Pathol* 1998; **152:** 1259–69.

16. Lecoin L, Gabella G, Le Douarin N. Origin of the c-kit positive intestinal cells in the avian bowel. *Development* 1996; **122:** 725–33.

17. Lee JR, Joshi V, Griffin JW Jr, et al. Gastrointestinal autonomic nerve tumor: immunohistochemical and molecular identity with gastrointestinal stromal tumor. *Am J Surg Pathol* 2001; **25:** 979–87.

18. Maeyama H, Hidaka E, Ota H, et al. Familial gastrointestinal stromal tumor with hyperpigmentation: association with a germline mutation of the *c-kit* gene. *Gastroenterology* 2001; **120:** 210–15.

19. Mazur MT, Clark HB. Gastric stromal tumors: reappraisal of histogenesis. *Am J Surg Pathol* 1983; **7:** 507–19.

20. Miettinen M, Lasota J. Gastrointestinal stromal tumors—definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001; **438:** 1–12.

21. Miettinen M, Monihan JM, Sarlomo-Rikala M, et al. Gastrointestinal stromal tumors/smooth muscle tumors/GISTs in the omentum and mesentery: clinicopathologic and immunohistochemical study of 26 cases. *Am J Surg Pathol* 1999; **23:** 1109–18.

22. Miettinen M, Sarlomo-Rikala M, Lasota J. Gastrointestinal stromal tumours: recent advances in understanding of their biology. *Hum Pathol* 1999; **30:** 1213–20.

23. Mudan SS, Conlon KC, Woodruff JM, et al. Salvage surgery for patients with recurrent gastrointestinal sarcoma: prognostic factors to guide patient selection. *Cancer* 2000; **88:** 66–74.

24. Nishida T, Hirota S, Taniguchi M, et al. Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. *Nat Genet*1998; **19:** 323–24.

25. Nishida T, Hirota S. Biological and clinical review of stromal tumors in the gastrointestinal tract. *Histol Histopathol* 2000; **15:** 1293–301.

26. Pidhorecky I, Cheney RT, Kraybill WG, et al. Gastrointestinal stromal tumors: current diagnosis, biologic behavior, and management. *Ann Surg Oncol* 2000; **7:** 705–12.

27. Plaat BEC, Hollema H, Molenaar WM, et al. Soft tissue leiomyosarcomas and malignant gastrointestinal stromal tumors: differences in clinical outcome and expression of multidrug resistance proteins. *J Clin Oncol* 2000; **18:** 3211–20.

28. Reith JD, Goldblum JR, Lyles RH, et al. Extragastrointestinal (soft tissue) stromal tumors: an analysis of 48 cases with emphasis on histologic predictors of outcome. *Mod Pathol* 2000; **13**: 77–85.

29. Rudolph P, Gloeckner K, Parwaresch R, et al. Immunophenotype, proliferation, DNA ploidy, and biological behavior of gastrointestinal stromal tumors: a multivariate clinicopathologic study. *Hum Pathol* 1998; **29:** 791–800.

30. Sakurai S, Fukusawa T, Chong J-M, et al. Embryonic form of smooth muscle myosin heavy chain (Seenb/MCH-B) in gastrointestinal stromal tumor and intestitial cells of Cajal. *Am J Pathol* 1999; **154:** 23–28.

31. Sakurai S, Hishima T, Takazawa Y, et al. Gastrointestinal stromal tumors and KIT-positive mesenchymal cells in the omentum. *Pathol Int* 2001; **51:** 524–31.

32. Sanders L, Silverman M, Rossi R, et al. Gastric smooth muscle tumors: diagnostic dilemmas and factors affecting outcome. *World J Surg* 1996; **20:** 992–95.

33. Strickland L, Letson GD, Muro-Cacho CA. Gastrointestinal stromal tumors. *Cancer Control* 2001; **8:** 252–61.

34. Vanderwinden JM, Rumessen JJ, De Laet MH, et al. CD34 immunoreactivity and interstitial cells of Cajal in the human and mouse gastrointestinal tract. *Cell Tissue Res* 2000; **302:** 145–53.

35. Wieczorek TJ, Faquin WC, Rubin BP, et al. Cytologic diagnosis of gastrointestinal stromal tumor with emphasis on the differential diagnosis with leiomyosarcoma. *Cancer* 2001; **93:** 276–87.

PROFORMA

C.I
Name
Age
Sex
Tobacco
Alcohol
Family History
Abd. Pain
Distension
Bleeding
Others
Endoscopy
Biopsy Number
HPR
IHC
CT/Usg
CXR
Metastasis
Site
Date of surgery
Type
Blood loss

Tumour spll
Complication
Biopsy number
HPR
Grade
Size
Mitosis
Necrosis
Margins
CKit
S100p
CD 34
Vimentin
SMA
Keratin / others
Adjuvant Treatment
Recurrence
Date of Last follow up
Condition